

RESEARCH ARTICLE

Isospora machadoae sp. nov. (Protozoa: Apicomplexa: Eimeriidae), a new coccidian species from white-necked thrushes *Turdus albicollis* (Passeriformes: Turdidae) of South America

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<http://zoobank.org/1958A69A-870C-43A7-8845-AFF87B9F40D2>

ABSTRACT. A new coccidian species parasitizing white-necked thrushes *Turdus albicollis* Vieillot, 1818 is described from the Parque Nacional do Itatiaia, in Southeastern Brazil. *Isospora machadoae* sp. nov. has oocysts that are sub-spherical, $22.2 \times 21.2 \mu\text{m}$, with bilayered wall, $\sim 1.3 \mu\text{m}$ thick. Outer layer is rough with micropyle and micropyle cap. Oocyst residuum is absent, but one or two polar granules are present. Sporocysts are ellipsoidal, $13.3 \times 9.7 \mu\text{m}$. The Stieda body is flattened to half-moon-shaped and substieda body rounded. Sporocyst residuum is present, composed of scattered spherules of different sizes. Sporozoites are vermiform with a refractile body and a nucleus. These parasitized thrushes had no apparent clinical signs of coccidiosis or high densities of oocysts in feces. This condition may be associated with a specific low pathogenicity of *I. machadoae* sp. nov. and/or with the conserved habitat of these birds, which ensures the ecological niches and thus the immunocompetence to wildlife.

KEY WORDS. Taxonomy, coccidia, oocysts, wild birds, Parque Nacional do Itatiaia.

INTRODUCTION

Brazil has a wide and diverse avifauna, with more than 1,900 species (CBRO 2014). Turdidae comprises 20 genera and 180 species, of which 87 are classified in the genus *Turdus* Linnaeus, 1758. Although passerines of this family occur on all continents except Antarctica, the vast majority of genera and species are found in New World (BirdLife International 2016).

White-necked thrushes *Turdus albicollis* Vieillot, 1818 are birds found in several South American countries (BirdLife International 2016). In Brazil is present at Atlantic Forest preferably

in moist and taller trees areas, and their habitat in the side of hills, but also found near the ground feeding on small fruits and insects. These birds are popular because of their presence with frequency in orchards, backyards and even in parks of cities, having a rich vocal repertoire (Sick 1997, Sigrist 2014).

Similarly to other vertebrates, birds can be parasitized by coccidia, which are predominantly protozoan parasites of the intestine, and may be associated with behavioral and physiological changes, morbidity and mortality in different species of birds (Dorrestein 2009). In wild birds, the presence of coccidia is common and therefore should not strongly impact their

health; however, in disturbed environments, the birds tend to be stressed and immunodeficient, and in these conditions the coccidiosis can be severe (Berto and Lopes 2013).

The coccidia have feco-oral transmission and can be identified and described by exogenous forms shed in the feces: the oocysts (Berto et al. 2014). The coccidian parasites described from birds of the Turdidae family in New World are (Berto et al. 2011, Cardozo et al. 2015): (1) *Isospora phaeornis* Levine, Van Riper & Van Riper, 1980 from *Myadestes obscurus* (Gmelin, 1789) (Levine et al. 1980); (2) *Isospora robini* McQuistion & Holmes, 1988 from *Turdus migratorius* Linnaeus, 1766 (McQuistion and Holmes 1988); (3) *Isospora tucuruensis* Lainson & Shaw, 1989; (4) and *Isospora albicollis* Lainson & Shaw, 1989 from *T. albicollis* (Lainson and Shaw 1989); (5) *Isospora zorzali* Keeler, Yabsley, Gibbs, McGraw & Hernandez, 2012 from *Catharus aurantirostris* (Hartlaub, 1850) (Keeler et al. 2012); and (6) *Isospora massardi* Lopes, Berto, Luz, Galvão, Ferreira & Lopes, 2014 from *T. albicollis* (Lopes et al. 2014).

The aim of this study was to examine the feces from white-necked thrushes *T. albicollis* to determine what coccidian parasites were present. These white-necked thrushes were captured in and around the Parque Nacional do Itatiaia, in Southeastern Brazil.

MATERIAL AND METHODS

Three expeditions were conducted in two localities in Southeastern Brazil: (1) Parque Nacional do Itatiaia, a protected area with a high degree of vulnerability, located in the Serra da Mantiqueira on the border of the States of Rio de Janeiro, Minas Gerais and São Paulo; and (2) Visconde de Mauá, which is a district near the Parque Nacional do Itatiaia boundary. Sampling occurred in March 2015 and October 2016 in the Parque Nacional do Itatiaia (22°27'38"S, 44°35'34"W), and March 2016 in Visconde de Mauá (22°19'46"S, 44°32'11"W). A total of eleven *T. albicollis* (seven from the Parque Nacional do Itatiaia and four from Visconde de Mauá) were captured. The birds were kept in individual boxes and feces collected immediately after defecation. After identification of the species, the bird was photographed and released and stool samples were placed in centrifuge tubes containing a potassium dichromate 2.5% ($K_2Cr_2O_7$) solution at 1:6 (v/v). Samples were carried to the Laboratório de Biologia de Coccídios, Departamento de Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro (UFRRJ). Samples were incubated at room temperature for 10 days or until ~70 % of the oocysts were sporulated. Oocysts were isolated by flotation in Sheather's sugar solution (Specific gravity: 1.20) and examined microscopically using the technique described by Duszynski and Wilber (1997) and Berto et al. (2014). Morphological observations, line drawings, photomicrographs and measurements were made using an Olympus BX binocular microscope coupled to a digital camera Eurocam 5.0. Line drawings were edited using two

software applications from Corel Draw Graphics Suite, version 11.0. All measurements are in micrometres and are given as the range followed by the mean in parentheses.

TAXONOMY

Fecal samples of four of the eleven white-necked thrushes examined had oocysts with morphotype still unreported in the scientific literature, and it is bellow described as a new species.

Isospora machadoae sp. nov.

<http://zoobank.org/1F9B7A3F-100D-45CE-8162-7E534A9DAAE5>
 Figs 1, 2

Diagnosis. Among all coccidian species recorded from Turdidae in the New World, only *I. machadoae* sp. nov. has an oocyst wall rough with micropyle and micropyle cap, which are very uncommon characteristic features in *Isospora* spp. from Passeriformes (Table 1). In addition, *I. machadoae* sp. nov. differed from others in having ellipsoidal sporocysts with Stieda body flattened to half-moon-shaped.

Description. Oocyst (n = 35) spherical to sub-spherical, 21–24 × 20–23 (22.2 × 21.2); length/width (L/W) ratio 1.0–1.2 (1.1). Wall bi-layered, 1.2–1.5 (1.3) thick, outer layer rough, with 2/3 of total thickness. Micropyle present with 7.0 wide. Micropyle cap present with slight protrusion, but barely discernible in some oocysts (Figs 1, 2). Oocyst residuum absent, but one or two sub-spherical, robust and refringent polar granules are present. Sporocysts (n = 26) 2, ellipsoidal, 12–14 × 9–11 (13.3 × 9.7); L/W ratio 1.2–1.5 (1.4). Stieda body present, flattened to half-moon-shaped, 0.5 high × 1.5 wide; sub-Stieda present, rounded, 1.5 high × 2.5 wide; para-Stieda body absent; sporocyst residuum present, composed of scattered spherules of different sizes. Sporozoites 4, vermiform, with a posterior refractile body and a nucleus.

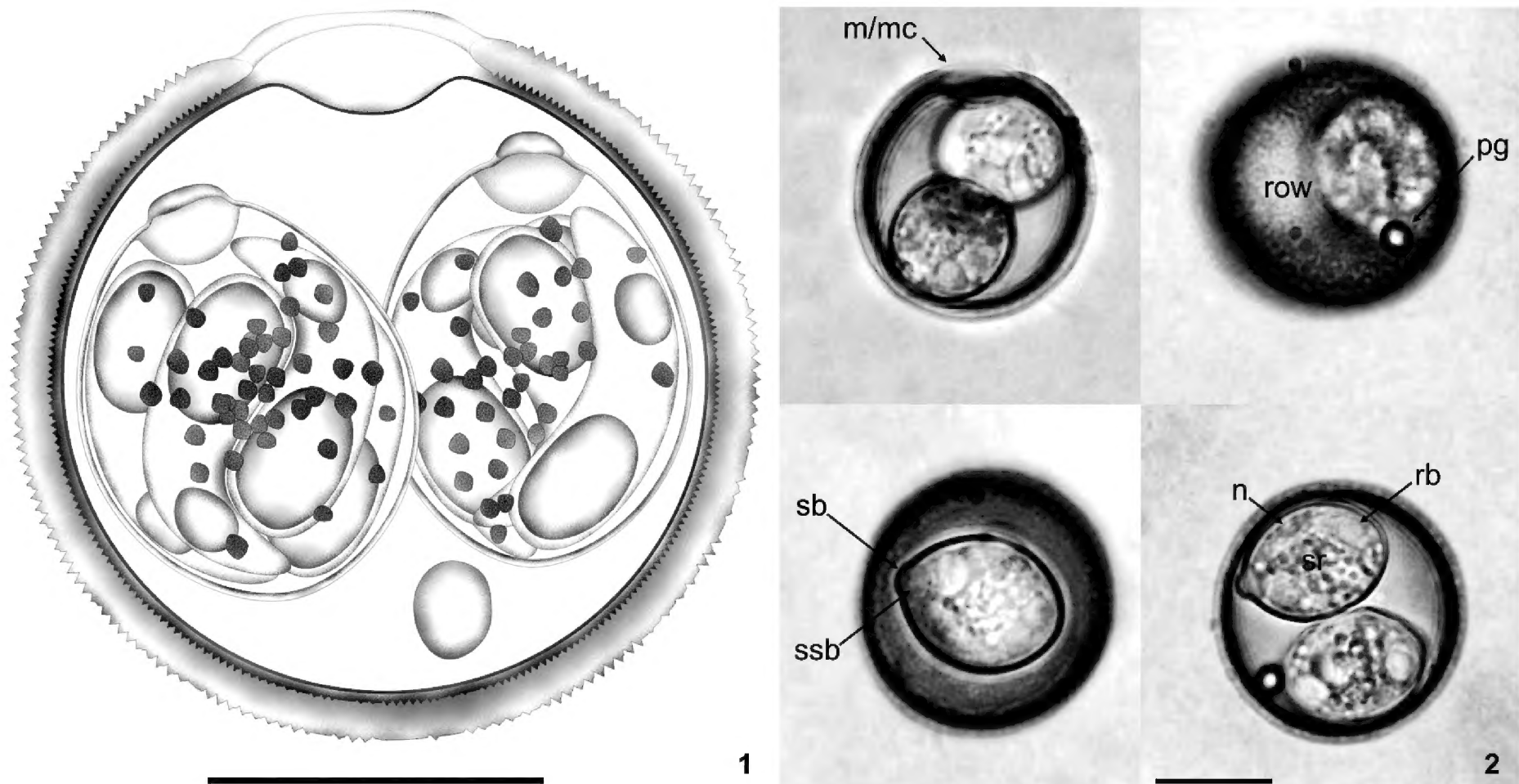
Type host. White-necked thrush *Turdus albicollis* Vieillot, 1818 (Aves: Passeriformes: Turdidae).

Type material. Phototypes, line drawing, half of the oocysts in 10 % aqueous buffered formalin (v/v) and the other half in 70 % ethanol are deposited at the Museu de Zoologia da Universidade Federal Rural do Rio de Janeiro, Brazil, under accession number MZURPTZ2017001. Phototypes and line drawings are also deposited and available (<http://r1.ufrj.br/labicoc/colecao.html>) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, under repository number P-70/2016. Photographs of the type-host specimen (symbiotype) are deposited in the same collection.

Type locality. Parque Nacional do Itatiaia (22°27'38"S, 44°35'34"W), Southeastern Brazil.

Other locality. Visconde de Mauá (22°19'46"S, 44°32'11"W), Southeastern Brazil.

Etymology. The specific name is derived from the family name of a Brazilian parasitologist Dr Rosangela Zacarias



Figures 1–2. Sporulated oocysts of *Isospora machadoae* sp. nov. recovered from *Turdus albicollis* from Brazil: (1) line drawing; (2) photomicrographs. Note the micropyle and micropyle cap (m/mc), nucleus (n), polar granule (pg), refractile body (rb), rough oocyst wall (row), Stieda (sb) and sub-Stieda bodies (ssb) and the sporocyst residuum (sr). Scale bar = 10 µm.

Table 1. Comparative morphology of *Isospora* spp. recorded from Turdidae of the New World.

| Coccidia | Hosts | References | Oocysts | | | | | | | | Sporocysts | | | | | | |
|-----------------------------|--------------------------------|-----------------------------|-------------------------|--------------|--------------|---------------|--------|-----------|---------------|--|-------------|--------------|-------------|---------------|---|-----------------------------|--------------------|
| | | | Shape | Length (µm) | Width (µm) | L/W ratio | Wall | Micropyle | Micropyle cap | Polar granule | Shape | Length (µm) | Width (µm) | L/W ratio | Stieda body | Substieda body | Residuum |
| <i>Isospora phaeornis</i> | <i>Myadestes obscurus</i> | Levine et al. (1980) | Ellipsoidal | 25–28 (27) | 18–20 (19) | – | Smooth | absent | Absent | Present | Ovoidal | 15–18 (16) | 10–11 (11) | – | Present | Present | Compact |
| <i>Isospora robini</i> | <i>Turdus migratorius</i> | McQuiston and Holmes (1988) | Ellipsoidal or ovoid | 20–28 (23) | 16–22 (20) | (1.2) | Smooth | absent | Absent | Present | Ovoid | 10–17 (13.8) | 7–12 (9.0) | (1.5) | Nipple-like | Present, prominent | Compact |
| <i>Isospora tucuruensis</i> | <i>Turdus albicollis</i> | Lainson and Shaw (1989) | Sub-spherical | 15–19 (17.3) | 14–19 (17.1) | – | Smooth | absent | Absent | Present, single, ~ 3 × 2 | Ellipsoidal | 10–13 (11.8) | 7–10 (8.4) | – | Nipple-like | Present, wide, ~ 0.5 × 1.5 | Diffuse or compact |
| <i>Isospora albicollis</i> | <i>T. albicollis</i> | Lainson and Shaw (1989) | Ovoidal | 22–27 (24.5) | 19–24 (20.3) | – | Smooth | present | Absent | Present, single, ~ 2.5 × 2 | Ellipsoidal | 12–15 (16.0) | 8–10 (11.2) | – | Nipple-like to bubble-shaped | Present, wide, ~ 1.0 × 4 | Diffuse or compact |
| <i>Isospora zorzali</i> | <i>Catharus aurantirostris</i> | Keeler et al. (2012) | Round to slightly ovoid | 16–24 (19.7) | 15–21 (18.6) | (1.1) | Smooth | absent | Absent | Present, 1 to 2 | Ovoidal | 11–18 (14.5) | 7–11 (8.5) | (1.7) | Nipple-like | Absent | Diffuse or compact |
| <i>Isospora massardi</i> | <i>T. albicollis</i> | Lopes et al. (2014) | Sub-spherical | 15–21 (18.6) | 14–19 (17.7) | 1.0–1.1 (1.1) | Smooth | absent | Absent | Present, 2 ellipsoidal Granules, 1.5 × 0.5 | Ovoidal | 13–16 (14.8) | 8–11 (9.3) | 1.4–1.8 (1.6) | Knob-like to rounded, 1 × 2 | Present, rounded, 1.5 × 3.5 | Diffuse |
| <i>Isospora machadoae</i> | <i>T. albicollis</i> | Current work | Sub-spherical | 21–24 (22.2) | 20–23 (21.2) | 1.0–1.2 (1.1) | Rough | present | Present | Present, 1 to 2, sub-spherical and robust | Ellipsoidal | 12–14 (13.3) | 9–11 (9.7) | 1.2–1.5 (1.4) | Present, flattened to half-moon-shaped, 0.5 × 1.5 | Present, rounded, 1.5 × 2.5 | Diffuse |

Machado, given in her honor for her contribution to the study of Protozoa.

Remarks. According to Duszynski and Wilber (1997) and Berto et al. (2011), a coccidian species should be compared in

detail with coccidian species that are feature-similar and belong to the same host family. The Turdidae is one of the families of Passeriformes with many descriptions of coccidian parasites, mainly considered the flycatchers, robins and nightingales

classified in the past as turdids. Currently, from the New World, only the six species cited in Table 1 are recorded from Turdidae (Berto et al. 2011, Cardozo et al. 2015). The white-necked thrushes captured in the current work had no apparent clinical signs of coccidiosis or high densities of oocysts in feces. This condition may be associated with a specific low pathogenicity of *I. machadoae* sp. nov. and/or with the good conservation status of the Parque Nacional do Itatiaia and Visconde de Mauá, which ensures the ecological niches and thus the immunocompetence to wildlife (Dorresteijn 2009, Berto and Lopes 2013).

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Author Contributions: IF, CWGL and BPB initiated and designed the study; IFP, LMS, MBR, BBL, MSO, HRL and BPB integrated the expeditions to capture wild birds and collect fecal samples; IF, HRL and BPB identified the birds species; IFP, LMS, MBR, BBL and MSO conducted the laboratory activities, including photomicrographs and measurements of the oocysts; BPB analyzed the data and drew the coccidian oocyst of the new species; IFP, CWGL and BPB wrote the paper.

Competing Interests: The authors have declared that no competing interests exist.